Synthesis of Zephycandidine A from Haemanthamine

Murphy, Paddy; Tibble-Howlings, Jamie; Kowalczyk, Radoslaw M. ; Stevens, Kevin

Tetrahedron Letters

DOI:
10.1016/j.tetlet.2020.151785

Published: 16/04/2020

Peer reviewed version

Synthesis of Zephycandidine A from Haemanthamine

Patrick J. Murphy* and Jamie W. Tibble-Howlings

School of Natural Sciences, Bangor University, Bangor, LL57 2UW, UK.

Radoslaw M. Kowalczyk

Chemical Analysis Facility, University of Reading, Reading RG6 6AD, UK.

Kevin Stevens

BioExtractions (Wales) Ltd., Unit 30, Tafarnaubach Industrial Estate, Tafarnaubach, Tredegar, Blaenau Gwent NP22 3AA, UK

Abstract: Zephycandidine A the first naturally occurring imidazo[1,2-*f*]phenanthridine alkaloid, isolated from *Zephyranthes candida* (*Amaryllidaceae*) has been prepared in three steps from the naturally occurring alkaloid haemanthamine.

Introduction

The isolation of zephycandidine A **1** in 2016[1] from *Zephyranthes candida* (*Amaryllidaceae*) represented the first report of a natural product containing an imidazo[1,2-*f*]phenanthridine nucleus. The structure of **1** was elucidated by a combination of spectroscopic analysis and predictive NMR calculations. (Fig. 1)

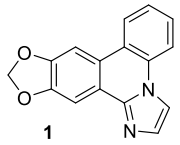


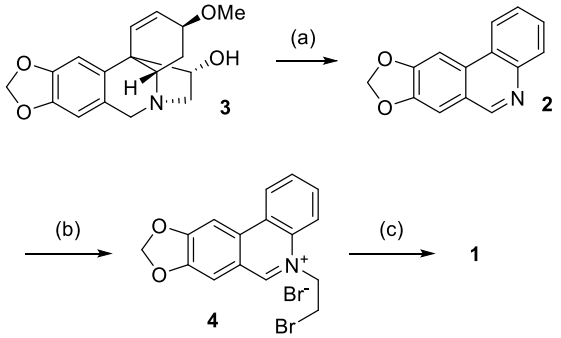
Figure 1: Zephycandidine A **1**

This metabolite exhibited significant cytotoxicity against five cancer cell lines, together with the ability to induce apoptosis in leukemia cells and displayed acetylcholinesterase (AChE) inhibitory activity. Our own interest in alkaloids from *Amaryllidaceae* lies in the isolation of alkaloids from species of daffodils and particularly with the use of waste-stream material generated from the commercial isolation of galanthamine. Galanthamine is currently used in the early stage treatment of Alzheimer's disease and similarly works by inhibiting acetylcholinesterase (AChE).[2]

Discussion

We were particularly interested in zephycandidine A **1** due to its structural similarity to the known[3] phenanthridine alkaloid trispheridine **2.** This metabolite was isolated[4] from *Zephyranthes candida*, and has been the topic of total synthesis by several groups.[5] We were particularly interested in the early report[6] of Warren and Wright who demonstrated that pyrolysis of the alkaloid haemanthamine **3** on a small scale led to the formation of **2** in good yield. We theorised that trispheridine **2** might be converted into zephycandidine A **1** and indeed the one-pot synthesis of imidazo[1,2-*f*]phenanthridine from phenanthridine reported by Cronin and co-workers[7] was suggested as a potential synthetic method by the group that isolated **1**.[1]

Access to daffodil waste-stream material from the isolation of galanthamine from Carlton daffodils[8] allowed us to access large quantities (>100 g) of haemanthamine **3**. Haemanthamine **3** was obtained by basification and extraction of an aqueous plant extract followed by recrystallization of the mixed alkaloids obtained from acetone.



Scheme 1: Synthesis of Zephycandidine A **1**. Reagents and conditions: (a) Heat, 190-195 °C, 24 h (see Table 1), 20 % (99% based on recovered **3**. (b) 1,2-dibromoethane, 90 °C, 7 days (c) (i) NH_3 (liquid), -78 °C to -33°C, 1h. (ii) MnO_2, $NaCO_3$, -78°C, 1h, then overnight warmed to rt. (iii) Toluene, reflux, 3h, 54 % (over 2 steps).

We attempted to repeat the work of Warren and Wright[6] (Scheme 1) who reported that by mixing **3** with powdered zinc and heating the mixture at an unspecified temperature, a high yield of **2** was obtained. In our hands, results on a small scale (100-200 mg of **3** and 3-40 equiv. of Zn Dust) were not encouraging. These gave either no conversion at lower temperatures (100 - 150 °C), partial conversion at 150 - 190 °C or complete decomposition at higher temperatures (195 - 220 °C). Reactions on a larger scale suffered from similar problems in that heating over commercially available zinc powder led to a poor conversion at 180-195 °C (Table 1, entries 1-4) whilst heating at 210-220 °C led to near complete decomposition. (Table 1, entry 5). Performing the reaction in air (Entry 1) or under nitrogen (Entry 2) or partial vacuum (Entry 3) had no apparent effects. In these cases the haemanthamine **3** was dispersed onto the zinc by evaporation from a methanol solution. Variations in the equivalents of zinc used were also not effective in improving the yield (Table 1, entries 1-3). Additionally the use of freshly activated zinc powder (Table 1, entries 6-7) gave little improvement, in these cases **3** was mixed with the zinc and ground to a fine powder. Considering a possible mechanism for this transformation (Scheme 2) we can visualise a fragmentation of **3** leading to the imine **5**, methanol and acetaldehyde enol. Tautomerisation of **5** leads to **6** which on oxidation, possibly by acetaldehyde leads to compound **2**. (Fig. 2)

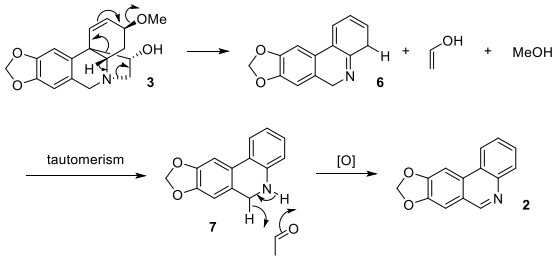


Figure 2: Possible mechanism for the formation of **2**.

This mechanism suggests that the zinc is superfluous to this reaction and we repeated the pyrolysis reaction in its absence. Thus, heating **3**in a sealed tube at 190-195 ºC for 24 h led to the formation of **2** in 20% yield together with recovered starting material in 79% yield (Table 1, entry 6). Repeating the pyrolysis for a longer time did not lead to a higher yield as considerable darkening of the reaction was observed after 2 d and complete decomposition was observed after 5 d. We dispersed **3** on sand to prevent direct contact with the oil bath and repeated the pyrolysis but again obtained a lower yield (entry 9). We next performed pyrolysis reactions in decalin at various temperatures and found no reaction at 175 - 180 ºC over 5 d (entry 10) and partial conversions at 180 - 185 ºC (entry 11) and at 190 - 195 ºC (entry 12) over 7 d. In the latter two cases decomposition was apparent and the amount of recovered starting material low. We also performed a reaction in which we added acetaldehyde as a potential oxidant (entry 13), however this gave similar yields and levels of decomposition. Whilst these reactions did not give the optimal conversion of haemanthamine **3** into trispheridine **2** previously reported,[6] heating **3** under the conditions of entry 8 followed by recycling of the recovered starting material gave reasonable quantities of **2** for the synthesis of **4**.

On heating **2** with freshly distilled 1,2-dibromoethane over 7 days a precipitate formed that was removed periodically to give **4** as an amorphous solid. The material yield for this process was good, however the product was contaminated with significant amounts (*ca*. 10-20%) of what was thought to be the hydrobromide salt of the starting material **2**. These two compounds were difficult to separate and only small quantities of pure **4** could be obtained by trituration in methanol. This by-product was thought to arise from decomposition of the reaction solvent leading to the formation of HBr. We attempted to prepare the salt at a lower temperature over a longer time, however this did not alleviate the problem. We decided to react this mixture under the conditions reported by Croninand co-workers[7] and thus it was added to liquid ammonia at -78 °C and stirred until completely dissolved. The mixture was then allowed to reflux (-33 °C) for 5 h, cooled to -78 °C and an excess of MnO_2 and Na_2CO_3 added. After warming to -33 °C over 1 h, the reaction was allowed to warm to rt overnight to evaporate the ammonia. At this point the residue was suspended in toluene and heated at reflux for 3 h. After filtration and evaporation, purification by silica gel chromatography gave Zephycandidine A **1** as an off white solid in 54 % yield together with the recovery of unreacted **2** from the previous step (Scheme 1).

Synthetic Zephycandidine A **1** gave NMR data close to that reported in the literature[1] when obtained at 400 MHz. This data was obtained in CD_3OD solution as used in the literature but due to varying amounts of HOD in the sample significant variations in chemical shift on standing and on reanalysis were observed. This might possibly have been due to small variations in the pH of the sample solution. Additionally as **1** was only sparingly soluble in CD_3OD we observed that without precise control of concentration and temperature very small variation in chemical shift occurred between different experiments. Reanalysis was subsequently performed at higher field (700 MHz) and the assignment of resonances from the proton and carbon NMR spectra for **1** are given in Table 2. The data agreed with the data reported previously[1] except for the protons at positions C-11 and C-12 which were misassigned in the original report. The HSQC spectrum of **1** indicates a clear correlation between chemical shift of the signal at 113.7 ppm (C-11) and the proton at 8.28 ppm and the signal at 131.3 ppm (C-12) and the proton at 7.50 ppm (Fig. 3). Synthetic **1** was fully soluble in a 10:1 mixture of $CDCl_3$ and CD_3OD and gave consistent NMR data. Full spectroscopic data and a comparison of NMR data for synthetic and natural **1** is available in the SI.

Conclusion

In conclusion, the *Amaryllidaceae* alkaloid zephycandidine A **1** has been independently synthesised for the first time from the alkaloid haemanthamine **3** via the alkaloid trispheridine **2**, confirming the proposed structure.

Acknowledgements

We thank Professor William B. Motherwell (UCL) for very helpful discussions, Dr Rolf Kraehenbuehl and Professor Bela Paizs for MS analysis, Professor Laurence M. Harwood and the University of Reading Chemical Analysis Facility for high resolution NMR, and Dr Loretta M. Murphy for assistance with UV analysis.

References

1) Zhan, G.; Qu, X.; Liu, J.; Tong, Q.; Zhou, L,; Sun, B; Yao, G. *Sci Rep*. **2016,** *6*, 33990.
2) Howes, M-J. R.; Perry, N. S. L.; Houghton, P. J. *Phytother. Res.* **2003**, *17*, 1-18.
3) Ghosal, S.; Saini, K. S.; Razdan, S. *Phytochemistry* **1985**, *24*, 2141-2156.
4) Luo, Z.; Wang, F.; Zhang, J.; Li, X.; Zhang, M.; Hao, X.; Xue, Y.; Li, Y.; Horgen, F.D.; Yao, G.; Zhang. Y. *J. Nat. Prod.* **2012**, *75*, 2113-2120.
5) a) Kumemura, T.; Choshi, T.; Yukawa, J.; Hirose, A.; Nobuhiro, J.; Hibino, S. *Heterocycles*, **2005**, 66, 87-90. b) Budén, M. E.; Dorn, V. B.; Gamba, M.; Pierini, A. B.; Rossi, R. A. *J. Org. Chem*. **2010**, *75*, 2206-2218. c) De, S.; Mishra, S.; Kakde, B. N.; Dey, D.; Bisai, A. *J. Org. Chem*., **2013**, *78*, 7823-7844. d) Borah, A.; Gogoi, P. *Eur. J. Org. Chem*. **2016**, 2200-2206. e) Harayama, T,; Akamatsu, H.; Okamura, K.; Miyagoe, T.; Akiyama, T.; Abea, H.; Takeuchia, Y. *J. Chem. Soc., Perkin Trans. 1*, **2001**, 523-528.
6) Warren, F. L.; Wright, W. G. *J. Chem. Soc.*, **1958**, 4696-4700.

7) Parenty, A. D. C.; Guthrie, K. M.; Song, Y-F.; Smith, L. V. Burkholder, E.; Cronin, L.; *Chem. Commun.* **2006**, 1194-1196.
8) a) van Zijl, P. C. M.; Ruessink, B. H.; Bulthuis, J.; MacLean, C. *Acc. Chem. Res.* **1984**, *17*, 172-180. b) Anet, F. A. L.; Kopelevich, M. *J. Am. Chem. Soc.* **1986**, *108*, 1354-1355. c) Alemany, L.B.; Gonzalez, A.; Billups, W.E.; Willcott, M.R.; Ezell, E.; Gozansky, E. *J. Org. Chem*. **1997**, *62*, 5771-5779.

Table 1: Preparation of trispheridine **2**.

Entry	Scale/ (mmol)	Temp./^{o}C	Zn/equiv.	Time/h	Yield **2**[i]/%	Recovered **3**/%	Decalin /mL	Sealed
1	16.6	180-90	36	3	10 (24)	59	0	No
2	19.1	190-95	72	24	4 (8)	52	0	No
3	6.6	190-95	185	24	11 (9)	55	0	No
4	16.8	190-95	72	24	11 (22)	50	0	No
5	16.9	210-20	72	7	7	0[ii]	0	No
6	8.3	190-95	75[iii]	24	11 (14)	22	0	No
7	8.3	160-90	75[iii]	48	10 (13)	25	0	No
8	1.7	190-95	0	24	**20 (99)**	80	0	Yes
9	5.0	190-95	0[iv]	24	11(39)	73	0	Yes
10	6.6	175-80	0	120	0	85	2	Yes
11	6.6	180-85	0	168	8 (15)	46	2	Yes
12	6.6	190-95	0	168	19 (22)	14	2	Yes
13	6.6	190-95	0[v]	24	13 (39)	33	2	Yes

i) Yields in brackets based on recovered **3**. ii) Considerable decomposition occurred. iii) Using freshly activated zinc. iv) **3** was dispersed on sand. v) 2 equiv. of acetaldehyde was added.

Table 1. Assignment of the NMR resonances for zephycandidine A **1**.

Position	^{13}C/ppm	^{1}H/ppm	*J*/Hz	Comments
1	125.21	8.39	dddd, 8.1, 1.2, 0.6, 0.6	Couplings to protons at C2, C3, C4 and C10
2	126.6	7.53	ddd, 8.1; 7.2; 1.2	
3	129.6	7.64	ddd, 8.2; 7.2; 1.2	
4	117.3	8.09	dd, 8.2; 1.2, 0.6	NOESY correlates protons at C4 and C11
4a	132.1			
6	143.6			
6a	119.5			HMBC correlates carbon at C6a to proton at C10
7	102.9	7.81	d, 0.6	
8	150.5			
9	151.4			
10	102.8	7.90	dd, 0.6, 0.6	
10a	125.23			
10b	122.9			
11	113.7	8.28	d, 1.5	NOESY correlates protons at C11 and C4
12	131.2	7.50	d, 1.5	
CH_2	103.5	6.15	d, 0.5	This residual dipolar coupling is a result of some molecules of **1** having preferential orientation in solution at 16.44T and it has been documented for aromatic molecules before.[8]

Figure 3: section of HSQC spectrum with the marked correlations at C11 and C12 positions.